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A cross sectional survey of health management and prevalence of vector-borne diseases, endoparasites and ectoparasites in Samoan dogs

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Abstract

Objective To determine prevalence of selected canine vector-borne diseases (CVBD) (*Leishmania infantum*, *Anaplasma* spp., *Ehrlichia canis*, *Borrelia burgdorferi* and *Dirofilaria immitis*), and endo- and ectoparasites in Samoan dogs presenting for surgical sterilisation, and to report on the general health management of the dogs.

Design and Procedure This study was a prospective serological cross-sectional survey. Management data were obtained for 242 dogs by interview with their owners. Blood samples were collected from 237 dogs and screened for the CVBD using point-of-care qualitative ELISA assays. *Anaplasma* spp. positive samples were screened by PCR and sequenced for

species identification. Rectal faecal samples were collected for faecal flotation from 204 dogs and immunofluorescent antibody tests were performed for *Giardia* and *Cryptosporidium* spp. on a subset of 93 faecal samples. The skin and coat of 221 dogs was examined for presence of ectoparasites.

Results *D. immitis* antigen was detected in 46.8% (111/237) of dogs. Seroprevalance of *Anaplasma* spp. was 8.4% (20/237); *Anaplasma platys* was confirmed by PCR. Prevalence of hookworm was 92.6% (185/205) and *Giardia* was 29.0% (27/93). Ectoparasites were detected on 210/221 (95.0%) of dogs examined and 228/242 dogs (94.2%) had never previously received any preventative medication.

Conclusions There is a very high prevalence of *D. immitis*, hookworm and external parasites in Samoan dogs, and prophylactic medication is rarely administered. This is the first report confirming *A. platys* in Samoa and the South Pacific islands. The public health implications of poor management of the dogs should be considered and investigated further.

Keywords

Anaplasma platys; *Dirofilaria immitis*; *Giardia*; hookworm; Samoa; canine

Abbreviations

CVBD, canine vector-borne diseases; HrCLM, hookworm related cutaneous larva migrans; IFA, immunofluorescent antibody test; PCR-RFLP, PCR-restriction fragment length polymorphism; RU, rural Upolu; UAA , urban Apia area.

Introduction

Samoa is situated in the South Pacific and has a tropical climate ideally suited to transmission of many vector-borne infectious diseases. Information on canine health and disease from the South Pacific islands, including Samoa, is sparse, with only one previous small scale serological survey investigating selected infectious diseases in dogs in Samoa[1]. The role of dogs as a reservoir for zoonotic diseases in Samoa is also unknown.

The Samoan dog type is a mixed breed, tending to be medium sized, with a short to medium hair-coat. The rate of dog ownership in Samoa is very high at 88%[2] and the vast majority of dogs are free-roaming, with no system of dog registration or licensing in the country. Large, free-roaming populations of dogs are known to increase the prevalence of canine disease and associated risk of zoonotic infection.[2,3] Dogs living within communities also defaecate in living areas of the towns and villages and this can increase the risk of zoonotic diseases such as hookworm-related cutaneous larva migrans (HrCLM)[4] and toxocariasis.[5] The condition of large populations of free-roaming dogs is often relatively poor and, globally, these dogs and their management comprise a major animal welfare issue.[6]

Canine vector-borne diseases (CVBDs) are a common cause of morbidity in dogs worldwide and the threat to human and animal health is disproportionately high in developing countries and tropical and sub-tropical regions.[7] Ticks in particular have been shown to be competent vectors of many important diseases.[8,9] Most of these diseases are only transmissible by certain tick species, and the range of the disease often mirrors that of the distribution of its tick vector. *Rhipicephalus sanguineus*, widely believed to be a vector of *Anaplasma platys* and a known vector of *Ehrlichia canis*,[10,11] has been confirmed to be present in Samoa.[1]

This study aimed to investigate the current canine preventative health status of Samoan dogs, the prevalence of *Dirofilaria immitis* and the seroprevalence of *Anaplasma* spp., *Borrelia*

burgdorferi, *E. canis*, and *Leishmania infantum* and the prevalence of selected external and gastrointestinal parasites.

Materials and Methods

Ethical approval

This study was approved by the Massey University Animal Ethics Committee.

Study population

This was a prospective cross-sectional survey of dogs presenting for surgical sterilisation at a series of free clinics held across both main Samoan islands over a total of eight weeks in July 2010 and August 2011. Dogs estimated to weigh over 10 kg and determined by a veterinarian to be healthy enough to be anaesthetised, were eligible to be included in the study. During the pilot study period in July 2010, the first five dogs to fit the above inclusion criteria each day were sampled until a total of 50 dogs was reached. During the August 2011 sampling period every dog that fitted the inclusion criteria was sampled. Owner consent was obtained for all owned dogs.

For each dog a questionnaire was completed by the owner. The questionnaire contained questions relating to the area in which the dog lived, the husbandry (indoor, free roaming), age and breed (if known) and any previous vaccination or antiparasitic treatments administered. For the purposes of statistical analysis the islands were divided into three areas based on the areas used by the Samoa Bureau of Statistics for the census:[12] the urban Apia area (UAA), rural Upolu (RU) and Savai'i (Figure 1).

Age was recorded when available, or was estimated by the attending veterinarian, and placed in one of four categories: less than 12 months old; greater than one year but less than two years old; greater than two years but less than three years old; and three years or older.

Blood samples

Jugular venous samples (10mL) were collected at the time of general anaesthesia into tubes containing EDTA and tubes without anticoagulant. Samples were immediately placed on ice and sera collected from the plain tubes. Both serum and EDTA samples were stored at -20°C within eight hours of collection, pending analysis following transport to the Institute of Veterinary, Animal and Biomedical Sciences, Massey University, New Zealand.

Faecal samples were collected directly from the rectum, where there was sufficient faeces on rectal examination, at the time of general anaesthesia. Faecal samples were stored at 4°C pending faecal flotation. Any faeces remaining after flotation had been performed were then stored at -20°C pending analysis for *Giardia* and *Cryptosporidium*, following transportation to New Zealand with blood samples.

A thorough examination for external parasites was also performed at the time of anaesthesia by a veterinarian or trained final-year veterinary student under supervision, where time allowed. The presence of fleas, lice or ticks was recorded, and the species identified and recorded where possible.

Blood and serum analysis

All ELISAs were performed at Massey University, New Zealand. Serum samples were thawed, brought to room temperature and centrifuged prior to testing. A commercially available point-of-care ELISA (SNAP 4Dx test kit, IDEXX Laboratories, Westbrook, Me) for the simultaneous detection of *Anaplasma* spp., *B. burgdorferi* and *E. canis* antibody and *D.*

immitis antigen; and a commercially available point-of-care ELISA (SNAP Leishmania test kit, IDEXX Laboratories, Westbrook, Me) for the detection of *L. infantum* antibody were used according to the manufacturer's instructions. All serum samples were processed within 60 days of collection.

For each sample that tested positive for *Anaplasma* spp. antibodies, DNA was extracted from 200 µL of EDTA-anticoagulated blood using a commercially available DNA extraction kit (DNeasy Blood & Tissue Kit®, Qiagen, Hilden, Germany) according to the manufacturer's instructions. Final elution volume was reduced to 50 µL. Previously described PCR amplifications of the partial 16S rRNA gene of *Ehrlichia* spp./*Anaplasma* spp. was performed.[13,14] Genomic DNA samples with appropriate amplicon size were sequenced and data compared with sequence data on GenBank using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Faecal analysis samples and ectoparasite screening

Faecal flotation was performed within 48 hours of collection by simple bench-top flotation technique for a qualitative worm burden analysis. Approximately 3-5 grams of faeces was mixed with a flotation solution (a saturated sodium chloride solution (NaCl, specific gravity 1.2) for samples obtained in July 2010 and a saturated sodium nitrate solution ($NaNO_3$, specific gravity 1.33) for samples obtained in August 2011), and strained to remove any large particles. The solution was transferred to a 15 ml test tube. Additional flotation solution was added, if required, to bring the volume up to 15 ml, and to create a positive meniscus onto which a coverslip was placed. The solution was allowed to stand for a minimum of 10 minutes. The coverslip was removed and placed on a glass slide for examination by light microscopy as described elsewhere.[15] The entire coverslip area was examined at 100x magnification, and 400x magnification was used to aid identification. Samples were

considered positive if one or more eggs were seen. The species or genus of parasite was recorded, including *Trichuris vulpis, Dipylidium caninum, Toxocara canis, Cappilaria* spp. and *Sarcocystis* spp. Hookworm eggs were identified just as 'hookworm', as visual identification of species is not possible without measurement.[16]

Following transportation to Massey University, New Zealand, direct indirect immunofluorescent antibody testing (IFA) was performed on any remaining faecal samples to detect *Giardia* and *Cryptosporidium* spp. cysts in faeces. After bringing the samples up to room temperature, approximately 1 g of faeces was homogenised with 700 µl of 1 x phosphate-buffered saline in a 1.5 ml tube, vortexed at high speed for 30 seconds and left to stand for 10 minutes. A 50 µl aliquot was taken from this sample, pipetted onto a microscope slide and placed into a 37˚C incubator until dry. To the slide, 50 µl of methanol was added and left to air dry for 10 minutes. The sample was then stained with 50 µl fluorescein isothiocyanate stain (Aqua-Glo G/C, Waterborne Inc), placed in a humidity chamber and incubated at 37˚C for 30 minutes. The slide was washed with 50 µl 1 x phosphate buffered saline, and covered with mounting medium and cover slip. Detection was completed using an ultraviolet epi-fluorescent microscope with scanning at 200x magnification. Samples were considered positive if one or more cysts were seen.

Statistical analysis

Prevalence was calculated along with 95% confidence intervals for the individual diseases, using a binomial exact confidence interval. A Fisher's exact test was used to test for differences in prevalence between geographical areas and age groups. This was chosen over a Chi-squared test due to the low number of positives in some groups. A P-value cut off of <0.05 was used to determine significance. Statistical analysis was performed using R (http://www.R-project.org/).

Results

Study population

A total of 242 dogs were included. Detailed results of the study population and serological, endoparasite and ectoparasite screening are shown in Table 1.

Dogs were included from all three regions and all dogs enrolled were the mixed breed type predominantly seen in Samoa. The median age was one year and 73% (176/242) of dogs were males. All dogs were reported to live outdoors; 230/242 dogs were owned and 12/242 were strays. Four percent (10/242) of dogs had ever previously visited a veterinary clinic, and nine of these were from the UAA, where the only veterinary clinic on Samoa is located, with the tenth dog from a nearby village. Nine of these dogs had visited for vaccination and deworming treatment, with the remaining dog having presented for an illness. Fourteen dogs had ever received an anthelmintic treatment, and six of these had received it within the previous six months. All but two of these dogs were from within the Apia urban area. Ten dogs were reported to have ever received a flea treatment, although most owners did not know what product had been used or when. Four dogs were reported to have been treated two to three months previously. For all other dogs it was either unknown or reported as "long ago". No dogs from Savai'i had ever been presented to a veterinarian, been vaccinated or treated with any anthelmintic or ectoparasiticide treatment.

SNAP 4Dx test and PCR results

Blood samples were collected from 237 dogs. The ELISA 4Dx test was positive for *Anaplasma* spp. antibodies in 20/237 (8.4%; 95% CI=5.2-12.7) of the dogs; and *D. immitis* antigen test was positive in 111/237 (46.8%; 95% CI=40.3-53.4) of the sample population;

there were no positive results for *B. burgdoferi, E. canis,* or *L. infantum* antibody, giving a prevalence of 0.0% (95% CI=0-1.5%) for each of these organisms. Thirteen of the 20 *Anaplasma* spp. seropositive samples were confirmed positive as *A. platys* following PCR screening and sequencing. Three isolates were confirmed *Wolbachia* spp.

There was a significant association between prevalence of *D. immitis* and both age and the area of Samoa the dog was from. There was a statistically significant difference (P<0.001) between the age categories, with older dogs having a higher prevalence of *D. immitis* than younger dogs. There were significant differences between all age categories except between the second and third, and the third and the fourth age categories. There was also a statistically significant difference (P<0.001) prevalence of *D. immitis* between all the areas (Figure 2). There was a statistically significant difference (P=0.009) in seroprevalence of *Anaplasma* spp. between the areas, with UAA and Savai'i having a higher seroprevalence than RU, although there was no significant difference between UAA and Savai'i.

Endo- and ectoparasite results

Of the 242 dogs enrolled on the study, 204 had faecal samples collected and analysed by faecal flotation. In the remaining dogs either the faecal sample obtained was too small to test or there were no faeces in the rectum to collect. Ninety-three of the collected faecal samples were then frozen, transported to New Zealand and analysed for *Giardia* and *Cryptosporidium* spp. at a later date. In the remaining 111 cases, the faecal sample collected was only large enough to complete faecal flotation, or was too liquid to transport. Faecal examination was positive for endoparasites in 190/204 dogs (93.1%) (Table 2). Of the 242 dogs in the study, 221 (91.3%) had a skin examination. External parasites were detected on 210/221 (95.0%) of dogs. Fleas were present on 185/221 dogs (83.7%) and lice were detected on 18/221 dogs (8.1%). The lice species identified was *Trichodectes canis*. Ticks were present on 93/221

dogs (42.1%) and where a positive identification was made, the species identified was *R. sanguineus*.. No *Ixodes* spp. ticks were confirmed.

Discussion

This study is the first to provide epidemiological data on vector-borne diseases of dogs in Samoa and the first known study to have demonstrated the presence of canine *A. platys* in the South Pacific region. The findings are important from both a veterinary and public health standpoint. CVBDs are increasingly a threat to human and animal health in both developing and developed countries.[7] By providing prevalence data on these infectious diseases, several of which are potentially zoonotic, this study contributes to the assessment of potential public health threats that the free-roaming dog population in Samoa might pose on local and tourist communities. This study also gives baseline prevalence levels for monitoring the results of any future preventative measures implemented.

The overall prevalence of *D. immitis* in dogs was 46.8% (111/237), a high prevalence for this parasitosis. The data from the questionnaire highlight the fact that heartworm prophylaxis in dogs in Samoa is very rare, and the tropical climate in Samoa is ideal for both development of the microfilariae and the maintenance of a mosquito vector population. Therefore a *D. immitis* prevalence rate of this magnitude could be expected. Prevalences ranging from 22.4-86% have been seen in previous studies in the Asia-Pacific region in countries with a similar climate and conditions; in a study conducted in Papua New Guinea, 86% of dogs were positive for heartworm disease on necropsy;[17] in New Caledonia prevalences of 22.4-66% have been reported in stray dog populations;[18] and in Hawaii a study confirmed a prevalence of 32% in dogs by microfilarial testing.[19] Dogs housed outside, as were all the Samoan dogs

in this study, have also been shown to be at increased risk of *D. immitis* infection, due to increased exposure to the mosquito vector.[20]

There was a significant difference in the prevalence of *D. immitis* between the different age groups, with sero-positivity increasing with age in the sample population. The highest prevalence rate of 66.7% (24/36) was seen in the oldest age category, dogs aged three years and over, and this pattern has been seen in previous studies[21,22] and is attributed to prolonged exposure of dogs to mosquitoes and a lack of prophylactic measures.[21] The age group with the lowest prevalence was dogs aged less than one year old, but for a disease with a pre-patent period of seven months, a prevalence of 18.9% (10/53) is still very high for this age group. The results of our study suggest that dogs are being exposed to infected mosquitoes from a very early age, and veterinarians must be aware that dogs as young as eight or nine months old might already be infected with *D. immitis*.

The presence of *Anaplasma* spp. has never before been documented in Samoa and data for countries elsewhere in the Pacific are sparse. In this study, the overall seroprevalence of *Anaplasma* spp. was 8.4% (20/237). PCR of positive samples confirmed *A. platys*, the predominant *Anaplasma* spp. in the Asia-Pacific region[14,23,24] and transmitted by *Rhipicephalus sanguineus*.

None of the 237 dogs tested were seropositive for *E. canis*, despite the high number of dogs in Samoa infested with its primary vector, *R. sanguineus.* The distribution of *E. canis* generally follows that of its vector, although some countries such as Australia appear to be free of *E. canis*, despite *R. sanguineus* being widespread throughout much of the country.[25] In a study into animal health in Samoa conducted in 1997 six out of 10 dogs tested positive for antibodies to *E. canis* (by IFA test).[1] This is at odds with the findings of the current study. The *E. canis* part of point-of-care ELISA test kit (SNAP 4Dx, IDEXX Laboratories,

Westbrook, ME) used in this study is calibrated to be positive at titres greater than 1:160[10] so at lower titres the test is less sensitive than IFA. However sensitivity and specificity are still excellent (96.2% and 100% respectively) when compared with the IFA.[26] Zero positive samples suggests either the organism is absent from Samoa, or the seroprevalence is very low, below 1.5%. The lack of positive samples for *E. canis* was an unexpected finding, and further testing using PCR or IFA would help to confirm if this was a true finding and not due to a problem with the test kit used or low antibody titres remaining undetected. It is possible that in the previous study[1] the IFA was cross-reacting with another *Ehrlichia* species. The IFA is known to cross-react with *Ehrlichia chaffeensis* and *Ehrlichia ewingii* resulting in false positive results, however the same could be true for the ELISA test.[27] It might also be possible that there was cross-reactivity with an *Anaplasma* species, such as *A. platys*.

There were no antibodies demonstrated to either *B. burgdorferi* or *L. infantum* in any dogs in this study, as would be expected for Samoa and other Pacific regions. The global distribution of *B. burgdorferi* follows that of its *Ixodes* spp. tick vectors, generally occurring in the northern hemisphere in temperate, cooler climates,[28] and never documented in Samoa. *Leishmania* tends to occur in warmer climates where a competent sandfly vector is present and there is no evidence that this exists in Samoa. In most maps showing the global distribution of *Leishmania*, Southeast Asia, Australia, New Zealand and the Pacific are markedly free of the parasite.[29]

This study demonstrated a very high level of intestinal parasitism in Samoan dogs, with faecal floatation tests positive in 93.1% (190/204) of samples. The vast majority of samples were positive for hookworm, with or without other parasites and the overall prevalence for hookworm was extremely high, at 90.7% (185/204). Hookworm infection is rarely documented at this high prevalence, but these results are comparable with an epidemiological

study of dogs from tea growing regions of India, which demonstrated a hookworm prevalence of 93% by conventional faecal flotation methods. The dogs in that study had similarly low levels of veterinary care with only 2% de-wormed in the previous six months.[16] The tropical climate in Samoa is ideal for the development and transmission of hookworm larvae, as is a large and dense roaming canine population.[16] The species of hookworm egg was not determined in this study, however an *Ancylostoma* spp. would be expected in a tropical climate.[30] Determination of the hookworm species would be a valuable extension of this study. An extremely high prevalence of hookworm infection in dogs could play a significant role in contributing to the incidence of HrCLM in human populations. The effect of hookworm on the Samoan or tourist population is hard to establish as there are no studies or reports in the literature on this topic.

Giardia was also shown to be endemic in Samoan dogs, with a prevalence of 29% (27/93). The parasite is found in dogs worldwide, and prevalence studies have varied from very low to as high as 55.2%, with higher prevalences tending to occur in studies examining shelter and kennelled dogs[31] and in younger populations.[32,33] Dogs can harbour *Giardia* spp. infections of both host-specific and zoonotic assemblages and the zoonotic importance of infection in dogs depends on the assemblages isolated. Determination of the *Giardia* assemblage requires PCR on DNA extracted from the faeces.[34] Further research into the assemblages carried by dogs in Samoa is needed to assess the zoonotic potential and the importance it might have to public health. Giardiasis is the most common parasitic infection affecting humans worldwide, with the majority of infections acquired by drinking contaminated water sources. Recommendations to prevent *Giardia* infection in humans include maintaining good personal hygiene and hand washing, especially when preparing food, avoiding contaminated drinking water and cleaning up and disposing of dog faeces.[35]

The range of other intestinal parasites in this study is similar to those reported in previous prevalence studies around the world.[33,36] However the prevalence rates in this study were surprisingly low given the relative absence of preventative veterinary care, with *T. vulpis* (6.9%, 14/204), *D. caninum* (4.4%, 9/204), *T. canis* (3.4%, 7/204), *Capillaria* spp. eggs (2.0%, 4/204) and *Sarcocystis* sporocysts (0.5%, 1/204) all detected. The low levels of *D. caninum* are surprising given that fleas, involved in the lifecycle, were detected on 83.7% (185/221) of dogs examined. A low detection rate could be the cause of this result, as faecal flotation has poor detection sensitivity for this particular parasite.[37]

Preventative healthcare of dogs in this study was very uncommon with only 14 dogs (5.8%) reported as ever having been de-wormed and only four of these (1.7%) within the last three months. Comparing these results with a questionnaire based study on attitudes towards dog management in Samoa[2] there are some similarities in the populations selected; 71% of dogs in that study were male, compared to 72.7% (176/242) in the current study and the geographical distribution of samples was fairly equivalent. However there were also some differences; 72% of dogs had never visited a veterinarian, compared to 95.9% (232/242) in the current study and 12% had ever been vaccinated, compared to 3.7% (9/242) in the current study. In the Farnworth study[2] 19% of dogs were already sterilised, suggesting that these dogs had already been presented to a veterinarian for sterilisation. The lower figures for previous veterinary care in the present study could indicate that by selecting dogs through a free mobile sterilisation clinic, as opposed to randomly, dogs are less likely to have had any prior veterinary care. These dogs could be more likely to have certain parasitoses, therefore prevalence might be higher in this study population than compared with that of the general Samoan dog population. People who regularly de-worm and vaccinate their dogs could be more likely to take their dog to the vet to be sterilised, rather than wait until a mobile clinic visits their village; these animals would have been excluded from this study.

Conclusions

The high prevalence of *D. immitis* means there is a significant risk of heartworm disease to the canine population of Samoa, and a need for veterinary awareness and owner education on the severity of this disease. The prevalence of canine dirofilariasis provides information for medical practitioners in order that *D. immitis* infection can be considered as a differential diagnosis for pulmonary nodules in humans.[38]

The presence of *A. platys* in Samoa provides useful information for veterinarians, and it should be considered a differential diagnosis in cases where thrombocytopaenia or bleeding symptoms are observed, but is should be considered of little zoonotic relevance.[3].

Given the high levels of intestinal and external parasitism, and canine heartworm infection detected in this study, preventative healthcare measures should be strongly recommended to dog owners in Samoa, although the associated costs are likely to preclude this for many Samoan dog owners. Greater preventative care would not only help to improve the health status of the dogs treated, but would also help to reduce the environmental burden of parasites, reducing the risk of infection being transmitted to other dogs, or potentially to humans where zoonotic disease is concerned. Further studies examining the effect of canine parasitism on the human population of Samoa are required.

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Table 1: The demographics and tests performed on the samples from 242 Samoan dogs. The number and percentage (%) of dogs sampled for each variable of interest are provided and number and percentage (%) of samples with a positive ELISA test result for heartworm antigen and *Anaplasma* antibody for each variable.

Variable		Total (%) n=242	Blood sample tested (%) n=237	Heartworm (%)	*Anaplasma* spp. (%)	Faecal sample examined (%) n=204	Skin examined (%) n=221
Age	<1 year	56 (23.1)	53 (22.4)	10 (18.9)	4 (7.5)	46 (22.5)	53 (24.0)
	≥1, <2 years	70 (28.9)	68 (28.7)	30 (44.1)	7 (10.3)	59 (28.9)	60 (27.1)
	≥2, <3 years	61 (25.2)	61 (25.7)	35 (57.4)	6 (9.8)	52 (25.5)	56 (25.3)
	≥3 years	36 (14.9)	36 (15.2)	24 (66.7)	3 (8.3)	31 (15.2)	33 (14.9)
	Not recorded	19 (7.9)	19 (8.0)	7 (36.8)	0 (0.0)	16 (7.8)	19 (8.6)
Sex	Male	176 (72.7)	171 (72.2)	88 (51.5)	17 (9.9)	149 (73.0)	161 (72.9)
	Female	64 (26.4)	64 (27.0)	22 (34.4)	3 (4.7)	53 (26.0)	58 (26.2)
	Unrecorded	2 (0.8)	2 (0.8)	1 (50.0)	0 (0)	2 (1.0)	2 (0.9)
Area	Urban Apia area	76 (31.4)	75 (31.6)	18 (24.0)	10 (13.3)	66 (32.4)	65 (29.4)
	Rural Upolu	110 (45.5)	109 (46.0)	54 (49.5)	3 (2.8)	89 (43.6)	102 (46.2)
	Savai'i	56 (23.1)	53 (22.4)	39 (73.6)	7 (13.2)	49 (24.0)	54 (24.4)
Lifestyle	Stray	12 (5.0)				5 (2.5)	12 (5.4)
	Owned	230 (95.0)				199 (97.5)	209 (94.6)
Previous	Ever visited vet	10 (4.1)				8 (3.9)	9 (4.1)
Veterinary	Ever vaccinated	9 (3.7)				7 (3.4)	8 (3.6)
Treatments	Ever dewormed	14 (5.8)				13 (5.9)	14 (5.8)

Figure 1: Map of Samoa marking the villages from which the 242 sampled dogs originated. The number of dogs sampled from each area is denoted by numbers in parentheses.

Figure 2: The prevalence of heartworm in 237 dogs in three main areas of Samoa, divided into age groups. There was a significant difference between the three areas ($P < 0.001$) when any effect of age was accounted for (ANOVA).

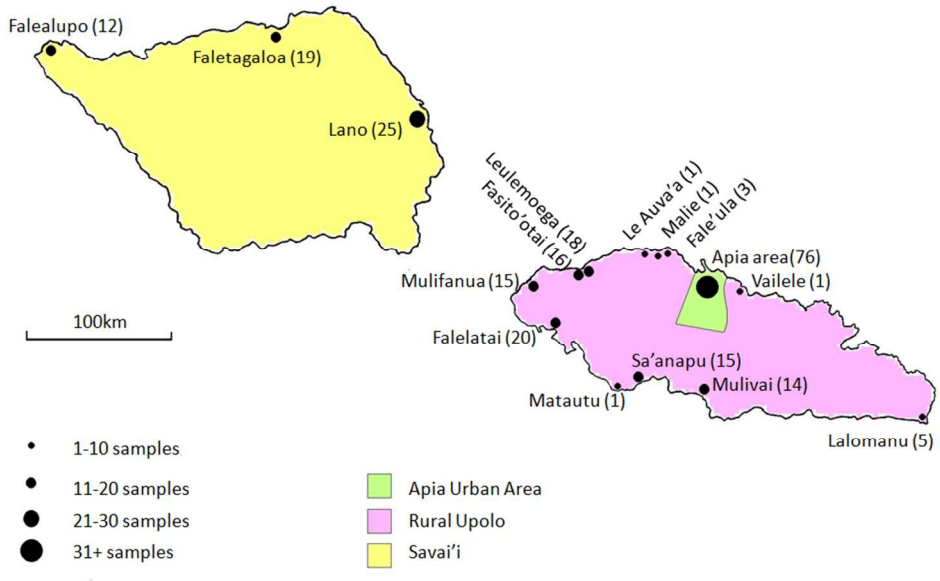

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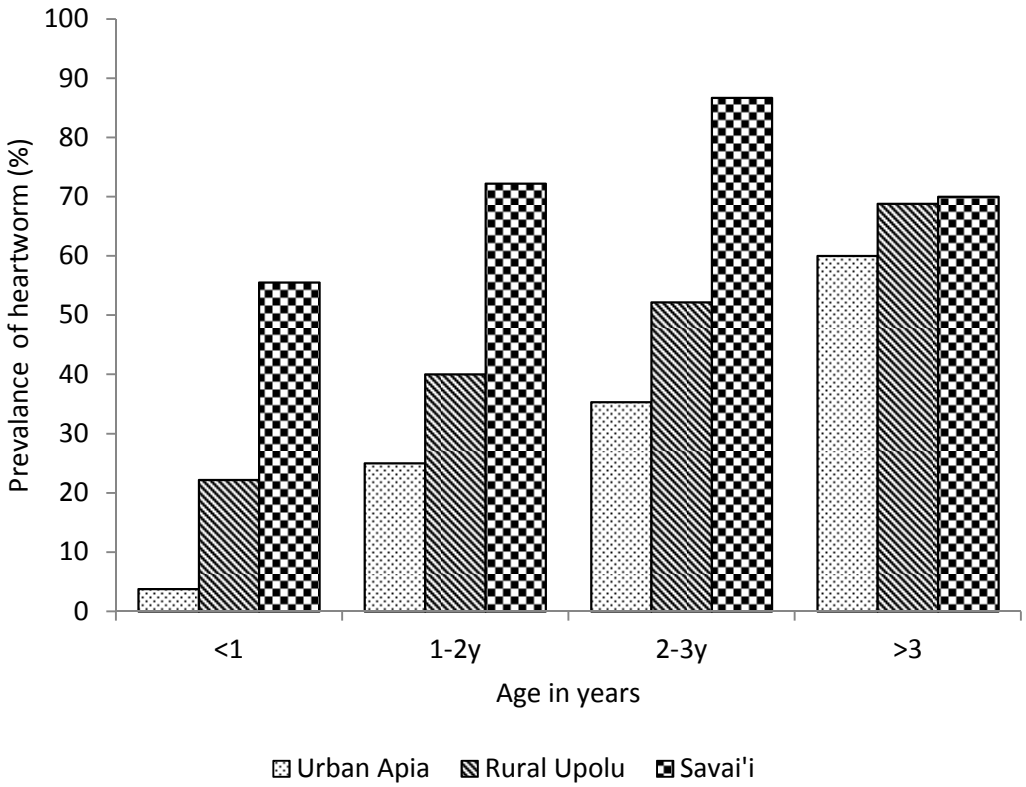
Figure 1


100
90
80
70
60
50
40
30
20
10
0
Prevalance of heartworm (%)
<1
1-2y
2-3y
>3
Age in years
Urban Apia
Rural Upolu
Savai'i